

THE PHYSIOLOGICAL RESPONSE OF ANTARCTIC FISHES TO ENVIRONMENTAL AND EXPERIMENTAL STRESS *

by

Stuart EGGINTON (1)

ABSTRACT. - From the relatively sparse data available, and on the basis of information from one group of fishes largely endemic to the seas around Antarctica (the red-blooded nototheniids), the stress response would appear to differ from that seen in other teleosts in both quantitative and qualitative aspects. There are unusually small changes in circulating catecholamine levels following induced stress. By contrast, there may be a substantial increase in haematocrit. In the absence of significant adrenergic stimulation this reflects an impressive polycythaemia, due to release of stored red cells from the spleen, rather than red cell swelling. Unlike other teleosts, splenic contraction, and other aspects of cardiovascular physiology, would appear to be largely under cholinergic control. Recovery times are similar to those observed in other fishes, suggesting effective rate compensation for the extreme low temperatures. A reduced glycolytic capacity is reflected in a dominance of respiratory, over metabolic acidosis. Unusually for marine teleosts, there may be an enhanced hypo-osmoregulation, although this occurs from a relatively hyper-osmotic baseline. The few data available for the haemoglobinless icefish suggests a similar down-regulation of adrenergic control, but with a greater degree of metabolic acidosis and a prolonged recovery period.

RÉSUMÉ. - Réponse physiologique des poissons antarctiques au stress de l'environnement et au stress expérimental.

A partir des rares données disponibles, et sur la base des informations provenant de l'un des groupes de poissons endémiques des mers qui entourent l'Antarctique (les Nototheniidae à sang rouge), la réponse au stress semble différente de celle des autres Téléostéens, à la fois d'un point de vue qualitatif et quantitatif. Les teneurs de catécholamine circulante varient exceptionnellement peu après un stress. En revanche, il y a un accroissement très net de la valeur de l'hématocrite. En l'absence de stimulation significative par l'adrénaline, cela reflète une impressionnante augmentation du nombre de cellules sanguines, surtout due à la libération des globules rouges stockés dans la rate. A l'inverse des autres Téléostéens, la contraction de la rate et les autres caractéristiques de la physiologie cardiovasculaire semblent surtout sous contrôle cholinergétique. La durée de rétablissement est semblable à celle des autres poissons, ce qui laisse supposer un réel taux de compensation aux très basses températures. Une capacité glycolytique réduite se reflète dans une acidose métabolique. De manière inhabituelle pour des Téléostéens marins, il pourrait y avoir un renforcement d'une hypo-osmorégulation, bien que celle-ci n'apparaisse qu'à partir d'un seuil relativement hyper-osmotique. Les rares données disponibles sur les poissons sans hémoglobine suggèrent un contrôle adrénérique analogue, mais avec une acidose métabolique plus élevée et une durée de rétablissement plus longue.

Key-words. - Nototheniidae, Channichthyidae, *Notothenia coriiceps*, *N. rossii*, *Chaenocephalus aceratus*, PSW, Antarctic Ocean, Physiology, Environmental stress, Experimental stress.

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(1) University of Birmingham, Department of Physiology, B15 2TT, U.K.

Any investigation into physiological adaptations must be aware of, and if possible quantify the effects of stress. These effects may vary according to the type and duration of stress (Pickering, 1981; Randall and Perry, 1992), and may be categorized according to their speed of recruitment. Primary stress responses refer to the initial changes to the autonomic nervous system and release of the stress hormones, catecholamines and corticosteroids. Secondary stress responses occur as a consequence of the biological action of these hormones, including changes in ionoregulation and acid base status, as well as altered cardiovascular function and cellular metabolism.

As experimental techniques have been refined, our understanding of the consequences of stress has changed our perception of what constitutes 'normal' values. The ever lower estimates of basal metabolic rate being a case in point. Nowhere has this been more evident than in the study of Antarctic fishes, where the physical isolation and logistical difficulties for sampling resulted in much of the early data being collected in our opportunistic, rather than systematic manner. It should come as no surprise that, in retrospect, much of this data was clearly obtained from highly stressed individuals.

The Antarctic fish fauna have been well characterized in terms of both traditional and molecular taxonomy (see other papers in this volume). In contrast, their physiology is a relatively unknown quantity. While the Southern Ocean offers a uniquely stable thermal environment within which cold adaptation of cellular function may be expected to have occurred, the physical isolation caused by the opening of the Drake Passage also provided the potential for development of unique characteristics as a result of endemic speciation (Eastman, 1993). It is sometimes difficult to distinguish between those features that reflect adaptation to cold, and specialisations of the notothenioids. Bearing in mind that virtually all physiological data has been gathered from this one group of fish, more interspecific comparisons are required before definitive conclusions may be reached.

MATERIALS AND METHODS

Yellowbelly rockcod, *Notothenia coriiceps* Richardson, Marbled rockcod, *N. rosii* Richardson and the haemoglobinless Blackfin icefish *Chaenocephalus aceratus* Lönnberg, were caught by trammel net to the north of the Antarctic Peninsula around the British Antarctic Survey's marine station on Signy Island (60°43'S, 45°36'W). This location is south of the Antarctic Convergence, the oceanographic feature delineating the region of endemic fish species, and well within their range of distribution. Experimental details are given elsewhere (Egginton, 1994, 1997).

Briefly, mixed venous blood samples were obtained by cardiac puncture from animals immediately after netting and during recovery for up to 96h. Similarly, the effects of anesthesia/surgery and forced exercise were determined from post-branchial arterial blood samples obtained via indwelling catheters. Respiratory gas tensions, pH and various parameters describing the hematology and blood chemistry were measured.

RESULTS AND DISCUSSION

One of the first problems that needs to be addressed is that of an appropriate capture technique. Most of the netting procedures in use are appropriate for systematics or biomass investigations, but provide specimens in a poor physiological state due to the often extreme acute stress. This may be particularly serious for the scaleless chan-

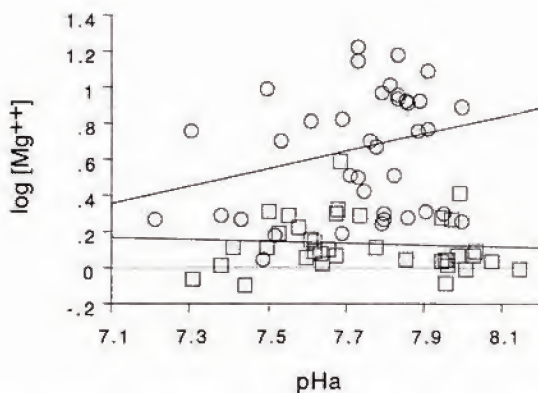


Fig. 1. - Plasma Mg^{2+} concentration for individual specimens of *Chaenocephalus aceratus* (circles) and *Notothenia coriiceps* (squares) subjected to various stressors (logarithmic scale). The intensity of stress is reflected in the arterial blood pH (pHa).

nichthyids, whose integument usually suffers damage by abrasion when held in nets. This probably explains the much greater range of plasma $[\text{Mg}^{2+}]$ found in the icefish, *Chaenocephalus aceratus*, compared with the heavily scaled nototheniid, *Notothenia coriiceps* (Fig. 1). Despite some overlap, two groups of icefish are represented: those whose renal handling of divalent cations is adequate to cope with any change in branchial permeability or increased drinking rates (low $[\text{Mg}^{2+}]$, lying below the regression line), and those

Table I. - Plasma catecholamine levels of Antarctic fishes (nmoles.L^{-1}).

	<i>Notothenia coriiceps</i>		<i>Chaenocephalus aceratus</i>	
	Noradrenaline	Adrenaline	Noradrenaline	Adrenaline
Effect of capture				
initial	2.49 ± 0.44	0.80 ± 0.20	1.44 ± 0.59	0.57 ± 0.30
t+3h	2.31 ± 0.18	1.03 ± 0.56	1.64 ± 0.40	0.42 ± 0.10
t+12h	2.23 ± 0.33	0.74 ± 0.22	1.15 ± 0.19	0.48 ± 0.25
t+24h	2.18 ± 0.01	0.94 ± 0.19	1.35 ± 1.29	0.81 ± 0.38
t+48h	0.89 ± 0.45	0.79 ± 0.55	1.12 ± 0.13	0.42 ± 0.10
t+96h	1.26 ± 0.33	0.54 ± 0.01	1.45 ± 0.84	0.68 ± 0.42
Effect of surgery				
initial	2.66 ± 1.81	0.72 ± 0.41	1.89 ± 1.15	0.50 ± 0.11
t+3h	-	-	2.35	0.50
t+12h	1.49 ± 0.47	0.58 ± 0.31	0.96 ± 0.52	0.48 ± 0.21
t+24h	1.18 ± 0.06	0.26 ± 0.14	1.83 ± 0.47	0.58 ± 0.04
t+48h	-	-	N/D	N/D
t+96h	1.36 ± 0.58	0.62 ± 0.29	0.77 ± 0.63	0.28 ± 0.25
	Rest		Exercise	
	Noradrenaline	Adrenaline	Noradrenaline	Adrenaline
<i>N. coriiceps</i>	1.49 ± 0.25	0.59 ± 0.06	1.46 ± 0.15	0.37 ± 0.10
<i>N. rossii</i>	1.65 ± 0.18	0.49 ± 0.06	1.64 ± 0.16	0.64 ± 0.05
<i>C. aceratus</i>	1.10 ± 0.48	0.44 ± 0.16	1.11 ± 0.36	0.19 ± 0.03

Mean \pm SEM; * $P < 0.05$ v. 96h samples.

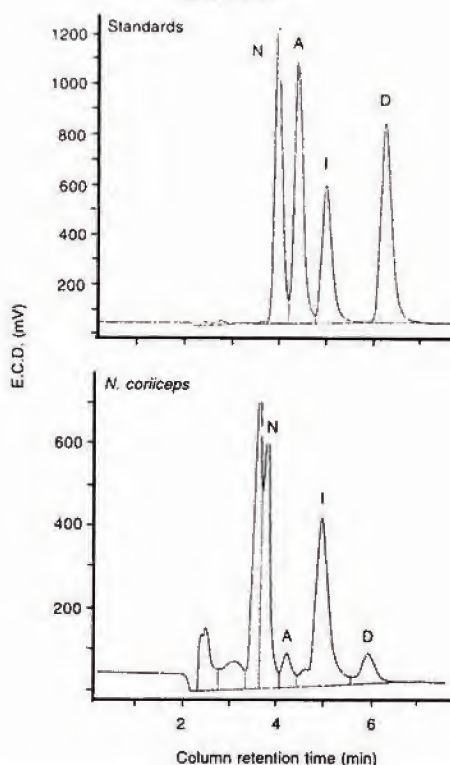


Fig. 2. - HPLC chromatograms using electrochemical detection of samples from *Notothenia coriiceps*, post-surgery. Standard elution profiles show noradrenaline (N), adrenaline (A), and dopamine (D), and an internal standard (I; 3,4 dihydroxybenzylamine).

whose ionoregulatory mechanisms are overwhelmed by the substantial ion influx across a damaged integument (high $[Mg^{2+}]$, above the regression line). Note that few of these were capable of withstanding large amounts of stress (low pHa).

The logistical difficulties, and cost, associated with conducting experiments in Antarctica is causing increasing pressure to use specimens returned to home institutions. The stress of transportation and exposure to different quality of water, type of food, temperature and photoperiod, is likely to have some effect. Davison *et al.* (1995) found higher heart rate and mean corpuscular hemoglobin concentration, and lower plasma $[Cl^-]$ in *Trematomus bernacchii* returned to New Zealand compared with specimens sampled at Scott Base in McMurdo Sound. Similarly, *N. coriiceps* sampled at Signy in the South Orkney Islands had higher blood pH and plasma $[Cl^-]$ and lower blood CO_2 tension and [lactate], than specimens returned to the United Kingdom (Egginton, 1997).

Although there are both quantitative and qualitative differences in reported values, reflecting the use of various stressors and species, it is clear that teleosts have an immediate and vigorous primary stress response. One of the most striking features of notothenioid physiology is that this is either lacking or severely blunted (Table I). While many other species appear to produce adrenaline as the dominant circulating catecholamine, noradrenaline titres were consistently higher in these fishes (Fig. 2). Similar concentrations of both catecholamines were reported for *T. bernacchii*, but again showed no change when subjected to handling or forced exercise (Davison *et al.* 1995). This unusually weak

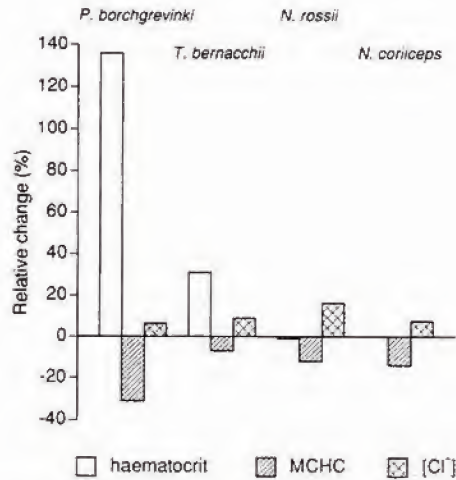


Fig. 3. - The effect of exercise stress on various nototheniids showing the relative change in haematocrit, mean corpuscular haemoglobin concentration (MCHC), and plasma chloride concentration $[Cl^-]$ (after Davison *et al.*, 1994; Egginton, 1997).

response cannot be interpreted as meaning the fish were not stressed, as changes in blood and plasma viscosity show (Egginton, 1996), but whether it reflects a failure of release or synthesis by the chromaffin tissue is unclear. Interestingly, injection of catecholamines elicits the usual increase in blood pressure and fall in branchial resistance, suggesting an intact receptor population (Egginton, 1997).

In view of the low hormone levels (above) it is of interest to observe the change in haematocrit (Hct) with stress. Teleosts usually show a pronounced increase in red blood cell volume (Na^+/H^+ induced swelling) and/or number (splenic contraction) when stressed, which facilitates oxygen transport, but is usually an adrenergic response. Although some nototheniid species do show the expected insensitivity of Hct, consistent with lack of catecholamine release, others do not (Fig. 3). It is clear that these differences do not reflect the lifestyle (pelagic or benthic) of the species concerned (Egginton and Davison, 1996), but rather the development of a cholinergic-dominated system of cardiovascular control (Nilsson *et al.*, 1996).

As may be expected for fishes that have undergone radiation in a stenothermal cold environment, there has been effective rate compensation in recovery from stress in nototheniids. Most hematology and blood chemistry parameters return close to baseline values within 12–24 h, although this is extended to around 48 h in the icefish, possibly reflecting the limited capacity for oxygen transport (Egginton, 1994; 1997). Of interest is the unusually low levels of blood lactate found in these fish (maximally around 4 mM). That this reflects a lack of metabolic production, rather than retention in skeletal muscle, was demonstrated by an unchanging intracellular [lactate] demonstrated by Davison *et al.* (1988) in red and white muscle of *Pagothenia borchgrevinki*. This phenomenon results in the unusual prevalence of respiratory over metabolic acidosis in nototheniids and although the icefish does show a more pronounced metabolic acidosis, it is still somewhat attenuated compared with temperate zone species (Egginton, 1994, 1997).

The latest unusual stress response to be reported is that of a hypo-osmotic reaction to chronic heat stress. In general, marine teleosts faced with any severe stress will be-

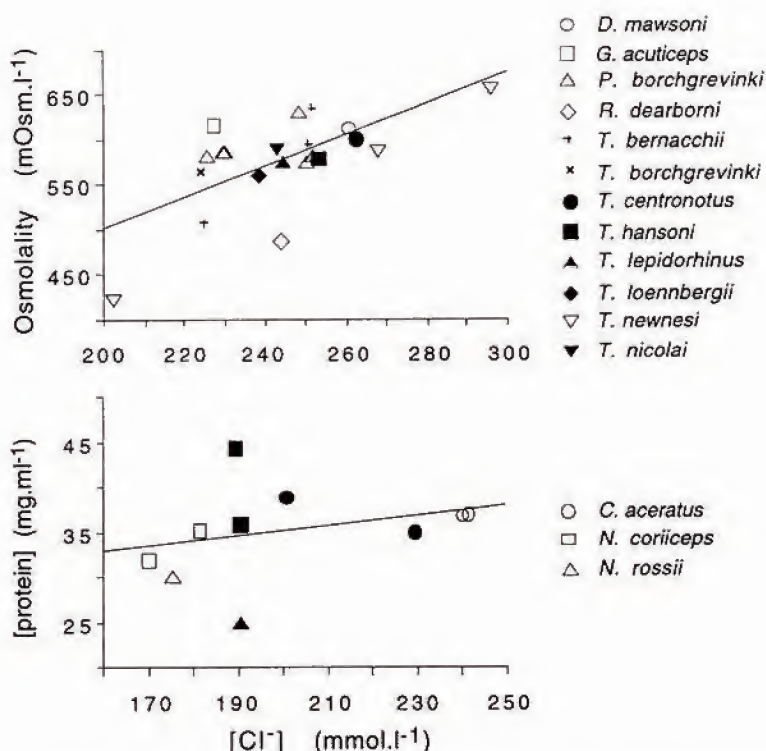


Fig. 4. - a) values for serum osmolality and [Cl⁻] reported in the literature for various notothenioids, b) plasma (protein) and [Cl⁻] for notothenioids and a channichthyid from Signy Island, subjected to various stressors (Egginton, 1994, 1996).

come hyperosmotic, due to increased branchial permeability resulting in water loss. However, Antarctic fishes are unusual in that their plasma osmolality is very high, possibly as an energy saving strategy, which is progressively (and reversibly) reduced after chronic exposure to temperatures close to their upper lethal limit (Gonzalez-Cabrera *et al.*, 1995). Why this should be so is at present unclear, and may simply reflect accentuated ion pump activity at higher than normal temperatures. There may be differences among species in their osmotic response, e.g. both *T. bernacchii* (Davison *et al.*, 1994) and *P. borchgrevinki* (Franklin *et al.*, 1993) showed the opposite effect to that seen by Gonzalez-Cabrera *et al.* (1995) following severe exercise and/or exposure to high temperatures. In addition, the control of ionic- and osmoregulation may vary. Reported values for serum osmolality cluster around 600 mOsm L⁻¹, while [Cl⁻] shows more variation (Fig. 4a). Moreover, while stressed notothenioids may increase plasma [Cl⁻], *C. aceratus* tends to decrease [Cl⁻] (Fig. 4b).

CONCLUSION

It can be seen that some unusual physiological responses to environmental and experimental stress are displayed by Antarctic fishes. We are, however, no better placed to decide which characteristics are phylogenetic (restricted to the endemic species) and which are adaptations to the extremely cold environment. More interspecific comparisons are required and, as the organizers of this workshop clearly envision, data from Arctic species would be particularly important, e.g., recent evidence for similar hyperosmotic regulation in *Eleginus navaga* (Christiansen *et al.*, 1995).

REFERENCES

- CHRISTIANSEN J.S., CHERNITSKY A.G. & O.V. KARAMUSHKO, 1995. - An Arctic teleost fish with a noticeably high blood fluid osmolality: a note on the navaga, *Eleginus navaga* (Pallas, 1811), from the White Sea. *Polar Biol.*, 15: 303-306.
- DAVISON W., FORSTER M.E., FRANKLIN C.E. & H.H. TAYLOR, 1988. - Recovery from exhausting exercise in an Antarctic fish, *Pagothenia borchgrevinki*. *Polar Biol.*, 8: 167-172.
- DAVISON W., FRANKLIN C.E. & J.C. MCKENZIE, 1994. - Haematological changes in an Antarctic teleost *Trematomus bernacchii* following stress. *Polar Biol.*, 14: 463-466.
- DAVISON W., AXELSSON M., FORSTER M.E. & S. NILSSON, 1995. - Cardiovascular responses to acute handling stress in the Antarctic fish *Trematomus bernacchii* are not mediated by circulatory catecholamines. *Fish Physiol. Biochem.*, 14: 253-257.
- EASTMAN J.T., 1993. - Antarctic Fish Biology, 322 p. San Diego: Acad. Press.
- EGGINTON S., 1994. - Stress response in two Antarctic teleosts (*Notothenia coriiceps* Richardson and *Chaenocephalus aceratus* Lönnberg) following capture and surgery. *J. Comp. Physiol., B*, 164: 482-491.
- EGGINTON S., 1996. - Blood rheology of Antarctic fishes: viscosity adaptations at very low temperatures. *J. Fish Biol.*, 48: 513-521.
- EGGINTON S., 1997. - Exercise-induced stress response in blood chemistry of three Antarctic teleosts. *J. Comp. Physiol., B*, 167: 129-134.
- EGGINTON S. & W. DAVISON, 1996. - Effects of environmental and experimental Stress on Antarctic Fishes. Soc. Exp. Biol. Seminar Srs., Cambridge Univ. Press (in press).
- FRANKLIN C.E., DAVISON W. & J.C. MCKENZIE, 1993. - The role of the spleen during exercise in the Antarctic teleost, *Pagothenia borchgrevinki*. *J. Exp. Biol.*, 174: 381-386.
- GONZALEZ-CABRERA P.J., DOWD F., PEDIBHOTLA V.T., ROSARIO R., STANLEY-SAMUELSON D. & D. PETZEL, 1995. - Enhanced hypo-osmoregulation induced by warm-acclimation in antarctic fish is mediated by increased gill and kidney Na⁺/K⁺-ATPase activities. *J. Exp. Biol.*, 198: 2279-2291.
- NILSSON S., FORSTER M.E., DAVISON W. & M. AXELSSON, 1996. - Nervous control of the spleen in the red-blooded Antarctic fish *Pagothenia borchgrevinki*. *Am. J. Physiol.*, 270: 599-604.
- PICKERING A.D., 1981. - Stress and Fish. 367 p. London: Acad. Press.
- RANDALL D.J. & S.F. PERRY, 1992. - Catecholamines, pp. 255-300. In: Fish Physiology, Vol. XIIb (Hoar W.S., Randall D.J. & A.P. Farrell, eds). San Diego: Acad. Press.

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